(19) Europäisches Patentamt
European Patent Offi
Offic uropéen des brev ts

(11) Publication number : **0 532 206 A1**

(12) # EUROPEAN PATENT APPLICATION

(21) Application number : 92307813.3

(22) Date of filing : 27.08.92

(51) Int. Cl.^{5} : **G01N 33/569**

Declaration under Rule 28(4) EPC (expert solution)

(30) Priority : 30.08.91 US 753270

(43) Date of publication of application : 17.03.93 Bulletin 93/11

(84) Designated Contracting States : AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

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(54) **Novel microorganisms discovered through unique process.**

(57) The subject invention pertains to novel microorganisms useful for the control of unwanted grasses and other weeds. The microorganisms of the subject invention are discovered through a unique process which involves isolating plant pathogens from asymptomatic plants.

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Background of th Inv nti n

B n ficial uses of microorganisms are well known in the art and hav been docum nted at great l ngth. Many patents have issued which claim new microbial process s pertaining to th production f antibiotics, n-zymes, thanol, and a multitud of ther useful products. Microorganisms ar also used t clean up toxic wastes and oil spills, kill pests, recover minerals, and provide nutrients to plants. It has been known for many years that some organisms produce compounds which are toxic to other organisms. The production of the antimicrobial compound penicillin by penicillium mold is one such example.

Microorganisms are particularly attractive candidates for use in making and delivering organic compounds because they can be extremely efficient and safe. The modern tools of genetic engineering have greatly enhanced the ability to exploit the efficiency and relative safety of microbes. Even in the absence of genetic manipulation, however, microbes can perform highly specific tasks which make them indispensable in certain applications. Thus, there is a constant ongoing search in many areas of research for previously unknown microbes with specific advantageous properties. The subject invention concerns the discovery of such microbes.

Weeds are a tremendous problem for farmers throughout the world. Weeds cause a 10-12% loss of value for agricultural products in the United States, the most recent estimate being $20 billion annually (McWhorter, C.G. [1984] Weed Sci. 32:850-855).

Undesirable grasses are a significant problem to homeowners, golf courses, and agriculture workers. Chemical control of these grasses may pollute the environment and often does not provide the necessary selectivity to kill pest grasses without harming desirable vegetation.

There exist multiple societal pressures for the replacement of chemical pesticides with alternate control methods. One area of active research along these lines involves the use of plant pathogens which can attack weeds. Although the existence of these pathogens is well known, and some of these pathogens have been patented, there are very few commercial products utilizing bioherbicides and these enjoy only limited use. For the most part, the organisms employed have been fungal pathogens with a much more limited effort having been directed towards bacterial pathogens. The process for finding such "bioherbicide" pathogens has a low success rate for yielding commercially applicable discoveries.

Microorganisms can be associated with plants in many ways. For example, some are saprophytic and some are pathogenic, but even those which are pathogens may exist only rarely in a phase of their life cycle or epidemiological event in which signs or symptoms of disease are evident. Many organisms which can be pathogenic under certain circumstances exist as epiphytes or endophytes on or in plants which are "healthy". These associations have been documented in the literature.

Some bacteria are known to infect certain grasses causing the grasses to be suppressed or killed. These infections have been known in various geographic locations as important problems for the maintenance of desirable grasses. A bacterial infection of Toronto creeping bentgrass which is used on golf putting greens is described by Roberts, D.L., et al. in Plant Disease 65, 1014-1015 (1981); Roberts, D.L., et al., Plant Disease 66, 804-806 (1982); Roberts, D.L., et al., Scanning Electron Microscopy IV, 1719-1722 (1983). The bacterium was identified as a Xanthomonas campestris by Roberts, D.L. in Phytopathology 73, 810 and 74, 813 (1984). The solution to the problem was treatment of the infection with oxytetracycline, an antibiotic. A disease of Poa annua L. was also described by Roberts, D.L. in Phytopathology 75 1289 (1985). The organism causing this disease has been deposited as NRRL B-18018 and is designated herein as MB218. In their 1982 paper, Egli and Schmidt described three new pathovars of Xanthomonas campestris, which caused wilt diseases of forage grasses. Previously all such wilt organisms were classified as X. campestris p.v. graminis, but by studying a large collection of isolates, these workers found some isolates with much narrower host ranges than the typical, graminis pathogens. One of these, p.v.poae, (ATCC 33804; MB238) was shown to cause wilting in only a few species of the genus Poa, most notably Poa trivialis or rough bluegrass.

Brief Summary of the Invention

The novel microorganisms of the subject invention have been discovered using a novel method for isolating microbes which are useful for the control of weeds. According to this method, microbes are isolated from asymptomatic plants. The microbes can then be grown and applied in greater concentrations to either the original host or other target weeds. Useful weed pathogens discovered using the method of the subject invention ar described herein.

Th pres nt inv ntion furth r relates t a m thod for controlling w d grasses by inf cting them with a Xanthomonas campestris pathovar which does not kilt non-w ed grasses. In on pref rred mbodiment of th inv ntion, th bacterial pathogens ben fit from direct acc ss to the xylem of the target weed. Access to th xyl m g nerally requires a wound to the target plant. In on sp cific mbodiment of the subject invention, w ed

grass s ar contr lled rapidly and effectiv ly with a treatm nt of a Xanthomonas campestris pathovar. Th control is facilitated by cutting the w d grass either bef r or aft r application of th bact ria.

The microb s of the subject inv ntion can also b transformed with genes coding for various toxins and th n re-applied to vegetati n. Th t xins may b , for example, from Bacillus thuringi nsis. Because of their ability to coloniz plants, th nov l microbes are us ful for deliv ry of the desired toxin.

Brief Summary of the Figure

Figure 1 compares the growth of three isolates at 6°C.

Detailed Description

The subject invention provides a novel method for isolating useful microbes. The microbes identified by the procedures described herein can be used to control weed grasses or other unwanted vegetation. In distinct contrast to methods previously used in this area of research, the plant pathogens identified according to the subject invention are isolated from asymptomatic plants. Thus, it is possible to take microbes which naturally co-exist with plants and use these microorganisms, in appropriate concentrations and under appropriate conditions, to control certain target weeds. The target weeds controlled by the plant pathogens may be the same plant the pathogen was isolated from or the plant may be different.

For purposes of this application, a "weed" is any plant that is objectionable or interferes with the activities or welfare of man. A "herbicide" (or chemical herbicide) is a chemical used to control, suppress, or kill plants, or to severely interrupt their normal growth processes. Herbicide Handbook of the Weed Society of America, Fifth Edition (1983), xxi-xxiv. As used herein, the terms "bioherbicide" and "microbial herbicide" mean a biological organism used to control, suppress, or kill plants, or to severely interrupt their normal growth processes.

The present invention further relates to a method for controlling a growing weed grass which comprises applying an infective amount of a Xanthomonas campestris pathovar to the weed grass whereby the weed grass is selectively suppressed or killed without suppressing or killing the non-weed grasses. A "weed grass" is a grass which is undesirable or interferes with the activities or welfare of man.

The cultures of the subject invention were deposited in the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Researçh Center, 1815 North University Street, Peoria, Illinois 61604 USA.

Culture	Accession No.	Deposit date
Xanthomonas campestris (MB 245)	NRRL B-18855	August 14, 1991
Xanthomonas campestris (MA 246)	NRRL B-18856	August 14, 1991
Xanthomonas campestris (MA 249)	NRRL B-18857	August 14, 1991
Xanthomonas campestris (MB 250)	NRRL B-18858	August 14, 1991
Xanthomonas campestris (MB 253)	NRRL B-18859	August 14, 1991
Xanthomonas campestris (MB 260)	NRRL B-18860	August 14, 1991
Xanthomonas campestris (MB 276)	NRRL B-18861	August 14, 1991
Xanthomonas campestris (MB 281)	NRRL B-18862	August 14, 1991
Xanthomonas campestris (MB 282)	NRRL B-18863	August 14, 1991
Xanthomonas campestris (MB 289)	NRRL B-18864	August 14, 1991
Xanthomonas campestris (MB 290)	NRRL B-18865	August 14, 1991
Xanthomonas campestris (MB 293)	NRRL B-18866	August 14, 1991

Th subject cultures hav b n d posit d under conditions that assure that access to th cultures will be

available during the p nd ncy of this patent application to n d t rmin d by th Commissioner of Patents and Trad marks to be ntitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. Th se deposits are available as required by foreign patent laws in counti s wh r in counterparts of the subject application, r its prog ny, are filed. How ver, it should b und rstood that th availability of a deposit does not constitute a licens to practice the subj ct invention in d rogation of pat nt rights grant d by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of a deposit, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent which may issue disclosing a culture. The depositor acknowledges the duty to replace a deposit should the depository be unable to furnish a sample when requested, due to the condition of a deposit. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

The invention can be used to control weeds in an agricultural setting and can also be used for nonagricultural applications. For example, the invention can be used for the control of weeds in turf and as a herbicide for the management of roadside vegetation.

An important aspect of the subject invention pertains to the use of wounds on the target weed which greatly enhance the activity of the bioherbicide. The use of wounds which, in and of themselves would not control the weed, is apparently very important for the proper establishment of the bacterial infection and for appropriate access of the active ingredients to the xylem of the plant. Thus, in a preferred embodiment of the subject invention, the microbial herbicide acts directly upon the xylem of the target weed. This direct access to the xylem is facilitated by, for example, a wound or other mechanical disruption of the weed's outer layers. This access to the xylem may also be achieved through the use of chemical means for compromising the integrity of the outer layers of the plant surfaces. For example, various enzymes can be used to enhance access to the xylem. In creating a mechanical wound, the target plant can be cut, nicked, sand blasted, mowed, etc. The microbial herbicide may be applied either before or after infliction of the wound. For example, the microbial herbicide may be sprayed ahead of a mower or behind a mower. Mowing after application of the microbial herbicide enhances the establishment of the microbial herbicide in the plant's vascular system. The effectiveness of this embodiment can be enhanced by surfactants (such as silicon surfactant) which facilitate movement of the bacteria into wounds or natural openings.

One of the most surprising aspects of the novel procedures used to isolate the plant pathogens of the subject invention is the discovery that pathogens of one grass species can be isolated from asymptomatic plants of an unrelated grass. This can be seen in the case of isolate MB260 which kills annual bluegrass but does not effect goosegrass, the plant from which it was isolated. Although the isolates discovered or studied here have been manipulated in order to have them function as "plant pathogens", their more normal ecological niche (and the setting in which they are found according to the subject invention) entails cryptic survival in plants. The discovery of these advantageous phytotoxic properties through the novel method of the subject invention is highly unexpected.

We have learned that the effective host range of a new bacterial isolate is not easily predicted based on the host of origin.

Compared to the p.v poae (ATCC 33804, MB238) described by Egli and Schmidt (1982), the isolates described here are much less pathogenic to rough bluegrass and much more pathogenic to annual bluegrass.

Our new isolates and pathovars, such as poae, do not have broad host ranges, but, they do cause a systemic wilt of certain unexpected species and some of these species are important, introduced, noxious weeds in the United States. Pathovar poae and many of the annual bluegrass pathogens can provide significant control of weedy species of the genus Bromus such as downy brome (Bromus tectorum). Pathogenicity of pathovar poae to Bromus has not previously been reported.

Crabgrasses (Digitaria sp.) are noxious, introduced weeds of importance in turf, but the literature does not describe any bacterial wilt disease of this host. Thus it was surprising to find that some of our annual bluegrass pathogens (MB276, MB266, MB269, for example) or other isolates have activity against that weed in wound-inoculation studies. This is another example of the benefits of our novel method of locating isolates since the best crabgrass pathogens in our collection came originally from healthy annual bluegrass samples.

Based on our observations described herein, we have discovered that there are many different organisms which are actually abundant and widespread, but whos normal ecological nich is not that f a pathogen, but more of a sub-l thal col niz r of plant xyl m. Thes bacteria do not produce bvious "dis as " in th field. The us of thes organisms as biocontrol agents relies on an artificial unbalancing of this symbiosis with the host plant. When the bacterium is grown t v ry high population levels in pure culture and inoculated into fresh wounds disease results. Th fact that th organism does not show a proclivity t wards autonomous epid mics

is actually an advantag in that th artificial use as a biocontrol ag nt is very unlikely to result in th initiation of dis ase except where applied int ntionally. Consid ring th infrequ ncy of "diseas " associat d with th natural persistence of thes bacteria, it is more appropriat to describe them as "xyl m ndophytes" rath r than as "plant pathogens". What is most important is that such rganisms, whatever th ir cological role, hav th utility of b ing manipulabl for the purpose of weed control.

In the case of organisms which are isolated from apparently healthy tissue, there is no basis for knowing the host range a priori. Some organisms which are pathogens in certain settings can quite effectively colonize plants without causing "disease;" therefore, if they are isolated from one host, it is impossible to predict which hosts, if any, they might be able to control by way of artificial inoculation. For instance, if Poa pratensis (Kentucky bluegrass) is inoculated with strains such as MB218, extremely high levels (>10^8 cfu/cm leaf tissue) of xylem colonization occur throughout the plant; however, the plant does not exhibit disease symptoms.

This phenomenon might be referred to as "non-host" colonization, but that designation simply reflects a classification system based on disease spectrum, not a realistic ecological understanding of the organism.

The novel organisms of the subject invention are useful for the control of undesired grasses in that when the target grasses are inoculated, the bacteria systemically colonize that plant and eventually lead to wilting and death. These bacteria are safe to use on most desired grasses either because the bacterium fails to colonize systemically or because colonization does not lead to wilting and death in those cases.

The bacteria of the subject invention can be fermented using a wide range of suitable bacterial media, can be dry stabilized or stored under refrigeration, and can be applied to the target grass using a wide range of spray equipment with the exception of CO_2 propellants. The formulation of the bacteria can include many carriers, and inert substances such as clays, microcrystalline cellulose and celite. The formulation may also comprise protectant compounds such as sugars, proteins and complex carbohydrates which are well known in the art. As described above, the application of the bacteria can be made with an accompanying injury to the target plants (mechanical or chemical) which will facilitate the colonization of the host by the bacteria.

The Xanthomonas campestris which is specifically exemplified herein can be applied as an aqueous solution or on an inert carrier. The solution or carrier preferably contains between about 10^6 to 10^{13} cells per gram (or ml for a solution. It will be appreciated that larger of smaller numbers of the cells per gram or ml can be used so long as infection and suppression of the weed grass is achieved.

Preferably the Xanthomonas campestris are provided for shipment to users in the form of a concentrate containing at least about 10^6 cells per gram or ml, and usually about 10^{13} per ml, which can be lyophilized to a greater concentration and mixed with a preservative agent, the exact composition of which depends upon the method of the preservation. The cells can be frozen or lyophilized. Where the cells are frozen, glycerol or various sugars and fresh growth media can be used as preservation agents. Amounts usually between 5 and 50% by volume of the glycerol or sugars can be used. Where the cells are lyophilized, nutrient media or various sugars can be used for preservation. Generally the Xanthomonas campestris, cells are grown to about 10^9 to 10^{11} cells per ml and may then be centrifuged or otherwise concentrated by removal of growth media. They can then be frozen or lyophilized or otherwise dried. As described above, the dried bacteria can be mixed with an inorganic solid carrier such as clays, talc, inert organic material or the like which may be dusted on the grasses or mixed with water and sprayed on the grasses.

All of these variations for storing, growing and applying the Xanthomonas campestris cultures are well known to those skilled in the art.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1: Isolation of Novel Microbes

Asymptomatic plants (plants which appeared to be completely healthy) including annual bluegrass, crabgrass, goosegrass and other species were collected from field, turf locations around the United States. Portions of these plants (leaves, stems, roots) were surface sterilized with either 10% bleach or 70% ethanol and chopped into small (1-2mm) pieces in water, saline, or phosphate buffer and incubated with gentle mixing for 30 minutes. The buffer or water was then plated onto suitable, bacteriological media which were either basically non-selective or broadly selective (Nutrient Agar, Modified Wilbrink's Agar (MWB) or MWB with cycloheximide, 5-fluorouracil, tobramycin and m thyl gr en - th latter m dium designed to reduce the numbers of th r bacteria and fungi recov red on th plates). By definition, it is not possibl t us a highly s lectiv medium because the antibiotic tolerance of an undiscov red organism cannot b known. After 2 to 10 days of growth at 22-38°C, individual colonies w r chosen from these plates and ith r directly inoculated to plants with needles r grown on solid or liquid media for subsequ nt inoculation using scissors r clipp rs.

Once these isolates had been successfully grown in some form of pure culture, they were inoculated to plants including the speci s from which th y were d rived and other species of interest. In the cases wh re desirabl activity was seen, the responsible organism was ither recovered from th inoculated plant r from a st ck cultur .

In summary, this method entails th ess ntially random wound inoculation scre ning of th int rnal microflora of healthy grasses with the screen being performed on the source species as well as on other species.

A list of organisms which were discovered using the method described above is presented in Table 1.

Table 1

Isolate code	Origin	Isolated from	Description: target and activity
MB250	New York	Asymptomatic Annual Blue-grass	ABG: virulent
MB246	Tennessee	Asymptomatic Annual Blue-grass	ABG: virulent
MB245	California La Jolla	Asymptomatic Annual Blue-grass	ABG: virulent, more active at lower temperatures
MB249	Texas, Dallas	Asymptomatic Annual Blue-grass	ABG: virulent, more active at lower temperatures
MB253	Texas, Tyler	Asymptomatic Annual Blue-grass	ABG: virulent
MB260	Missouri	Asymptomatic Goose Grass	ABG: virulent
MB251	Alabama	Asymptomatic Crabgrass	Crabgrass: leaf injury but not wilting
MB263	Alabama	Asymptomatic Annual Blue-grass	ABG: low virulence
MB264	Kentucky	Asymptomatic Annual Blue-grass	ABG: low virulence
MB281	Louisiana	Asymptomatic Annual Blue-grass	ABG: virulent
MB282	Florida	Annual Bluegrass	ABG: virulent
MB266	Louisiana	Asymptomatic Annual Blue-grass	Crabgrass: low virulence
MB293	Colorado	Asymptomatic Annual Blue-grass	ABG: virulent
MB289	South Dakota	Asymptomatic Annual Blue-grass	ABG: virulent
MB290	Washington	Asymptomatic Annual Blue-grass	ABG: virulent
MB276	California, Rancho Sante Fe	Asymptomatic Annual Blue-grass	ABG: virulent, superior activity against perennial biotype Crabgrass: moderate virulence

Additional studi s w re conducted with thes n w isolates with th hope of finding useful variability within th now xpand d collecti n. Thes experim nts includ d t mp ratur toleranc and host-range comparisons.

Exampl 2: A comparison f the virul nc f isolates und r cool conditions

Three isolat s of Xanthomonas (MB249, MB245 and MB218) w re us d to inoculate shak flask cultures (thre separate flasks per isolat). Th s w re grown at 22°C for 72 hours and then used f r inoculation of annual bluegrass seedlings. Th s parate flasks were also numerat d by dilution plate counting n Nutrient Agar. See Table 2. The inoculated plants were incubated in growth chambers with either 12 hour days at 85°F, 65°F or 55°F and 12 hour, nights at either 65°F, 45°F or 35°F respectively. Ratings of weed control were made over time. The results of these experiments are shown in Table 3.

The bacterial populations used for inoculation were very similar:

Table 2

Isolate	Flask Number	Population (cfu/ml)
MB218 Michigan	1	$4X10^9$
	2	$1x10^9$
	3	$2x10^9$
MB245 California	1	$3X10^9$
	2	$3X10^9$
	3	$3X10^9$
MB249 Texas	1	$2X10^9$
	2	$3X10^9$
	3	$2X10^9$

The California and Texas isolates caused a more rapid decline of annual bluegrass at cool temperatures although they initiated a similar level of disease at warmer temperatures:

Tabl 3

T m p.	Isolat	P rcent control f Annual Bluegrass								
		Day 9	Day 13	Day 15	Day 20	Day 26	Day 36	Day 41	Day 53	Day 62
85°F /65°F (war m)	MB218	70	80	99	100	100	100	100	100	100
	MB245	70	90	99	100	100	100	100	100	100
	MB249	75	95	99	100	100	100	100	100	100
65°F /45°F (coo l)	MB218	0	0	30	65	75	95	100	100	100
	MB245	0	60	75	85	90	99	100	100	100
	MB249	0	40	70	85	90	99	100	100	100
55°F /35°F (cold)	MB218	0	0	0	0	0	0	<	37	48
	MB245	0	0	0	0	0	25	45	80	85
	MB249	0	0	0	0	0	15	25	83	85

Although the annual bluegrass isolates in Table 1 have proven to be very similar in extensive host range studies and in biochemical characterizations, some virulence differences have been observed as noted in Tables 1 and 3. Two isolates were able to provide control of annual bluegrass at low temperatures. The ability to control weed grasses at low temperatures is a critical attribute because it is very desirable to control annual bluegrass, a winter annual, early in the season before it produces seedheads and before it can become established as large spots in the turf.

The temperature response differences which have been detected between isolates are large enough to establish that these are separate strains. The most virulent strains under cool conditions are MB249 and MB245. We have studied the plasmid profiles of these strains and using that method, it is possible to distinguish them from any previously reported strains. Strain MB218 contains a single plasmid (approximately 35 Kb) with 3 bands after digestion with EcoRI. Strain MB249 contains three similar bands and two new bands after EcoRI digestion. Strain MB245 contains no plasmid. This difference is useful for showing that these are distinct isolates.

Only by conducting the asymptomatic plant discovery program, described and claimed here, was it possible to sufficiently expand the base of isolates in order to demonstrate an underlying variability among isolates relative to cool temperature virulence.

Example 3: Host range of *Xanthomonas* isolates

Broth cultures of various Xanthomonas isolates were grown in either nutrient broth or Modified Wilbrink's medium for 72 hours at room temperature. These were diluted, if necessary, to achieve an approximate cell concentration of 10^9 cfu/ml. Scissors, alcohol sterilized and flamed between isolates, were dipped into the cell suspensions and used to cut seedling plants of various species. The plants were incubated in the greenhouse and obs rved for symptoms. The results are presented in Tables 4 and 5.

Tabl 4: Susceptibility among Bromus species (Ratings: + + > 70% control; + 30-70% control; -n control bserv d in this test)

Target species	MB218	MB249	pathovar graminis	pathovar poa
fasiciculatus	++	++	-	++
sericeus	++	++	-	++
hordeaceus	++	++	-	++
madritensis	++	++	-	++
scoparius	++	++	++	++
tectorum	++	++	++	++
antolicus	++	++	+	++
danthoniae	++	++	+	++
oxtodron	++	++	+	++
erectus	_	+	-	++
alopecurus	-	+	-	++
secalinus	-	-	++	-
rubens	-	-	-	+
anomalus	-	+	_	_
pseudodanthoniae	++	++	-	++
sewerzovii	-	-	++	-
mollis	+	+	-	+
squarrosus	-	+	+	-
rigidus	+	++	-	+
macranthos	-	+	-	
arduennsnsis	-	+	-	-

Table 5; Susceptibility of crabgrass to Xanthomonas isolates

Isolate	Percent Kill of Crabgrass	Percent kill of annual bluegrass
MB276	44	100
MB218	20	100
MB249	0	100
MB282	3	100

Example 4: Low temperature fermentation comparison

Three isolates of Xanthomonas campestris were aseptically inoculated to Nutrient broth and grown in shakeflasks in a temperature controlled shaking incubator at a constant 6°C. At intervals after inoculation, the growth of the cells was monitored bas d n light scattering by measuring the absorbance at 640 nm using a Specronic-20 sp ctrophotometer. Und r th s conditions th strains MB245 and MB249 achieved higher cell d nsities more rapidly than did strain MB218 (Figure 1). Mor rapid achi v ment of high c ll densities has also b n noted at warmer temp ratures in shake flasks and in f rm nt r. Thus, th s strains show superior char-

acteristics for growth in liquid cultur at less than or equal to room temperature.

Exampl 5: Increased turfgrass saf ty

Isolates of Xanthomonas campestris wer grown for 72 h urs at room t mp ratur in modifi d Wilbrink's broth and used for inoculation of a wide range of seedling plantings of turfgrass cultivars. The cell density of the broths was monitored by standard, dilution plating methods to insure saturation inoculum levels for a host range test. Plants were then incubated in the greenhouse and monitored for systemic wilt symptoms characteristic of infection by these pathogens. Positive controls of susceptible species (annual bluegrass) were included with each test as were comparison inoculations with known pathovars of any grass species being tested. The vast majority of desirable turfgrasses were not at all susceptible to any of the Xanthomonas isolates, but in some cases specific cultivars showed differential susceptiblility to the different isolates. A summary of these observations are listed in Table 6 below. The susceptibility of a cultivar is rated as "slight" if occasional seedlings demonstrate wilt symptoms. The susceptibility is listed as "moderate" if between 20 and 50 percent of the seedlings wilt. The susceptibility is listed as "high" if most of the seedlings wilt.

Table 6

Turfgrass Species	Cultivar	Level of Susceptibility to:	
		MB218	MB245
Creeping red fescue	'Dawson'	slight	none
Perennial rygrass	'Derby'	slight	none
Kentucky bluegrass	'Midnight'	moderate	slight
	'Ram I'	high	slight to moderate
	'Tendos'	high	slight

The low rates of injury to desired turfgrasses caused by the Xanthomonas isolates makes it possible to control weed grasses which are mixed in with turfgrasses without harming the desired turfgrasses. Thus, the isolates of the subject invention may be safely applied to turfgrasses for the control of, for example, annual bluegrass, Bromus species, Digitaria species, and Poa annua var. reptans.

Example 6: Accelerated control of annual bluegrass using a combination of *Xanthomonas campetris* isolates and a plant growth regulator, mefluidide.

Small pots of greenhouse grown, seedling annual bluegrass were sprayed with Xanthomonas cell suspensions of two different strains: MB245 (4×10^9 cfu/ml) and MB218 (3×10^9 cfu/ml) alone or in combination with mefluidide (Embark™) at rates of.03 or.15%. Control plants were sprayed with water or the same rates of mefluidide used in the combinations. Plants were approximately 6 cm tall when sprayed with the suspensions/solutions at the rate of 100 gallons per acre. While still wet with the spray material, they were cut to a heigt of 5 cm. The plants were then placed in a growth chamber with 12 hour, 45°F nights and 12 hour 65°F days. The effect of the treatments were quantified 10 and 14 days later by measuring the fresh weight of all grass above the soil line (Table 7). Reduced weight is attributable to the effects of the growth regulator or to the wilting effect of the bacterium, depending on the treatment.

Tabl 7: Accelerated control using Xanthomonas in combination with th plant growth regulator, m fluidide

Bact rium	Mefluidide concentration	Fresh w ight 10 DAT (grams)	Fresh weight 14 DAT (grams)
–	–	1.01	8.04
MB245	–	0.64	0.58
MB218	–	1.22	2.02
–	0.03%	0.65	6.26
–	0.15%	0.46	1.42
MB245	0.03%	0.28	0.38
MB245	0.15%	0.25	0.11
MB218	0.03%	0.42	0.98
MB218	0.15%	0.24	0.12

These data demonstrate that the advantageous herbicidal effect of the bacteria can be enhanced and/or accelerated by the use of plant growth regulator. A variety of growth regulators are known to those skilled in this art and could be used according to the teachings provided herein.

Claims

1. A method for identifying and isolating novel microorganisms that are pathogenic to target weeds, the process comprising
   (i) isolating microorganisms from an asymptomatic plant;
   (ii) growing the isolated microorganisms, to produce a stock solution;
   (iii) inoculating weed species of interest with microorganisms obtained in steps (i) and (ii);
   (iv) observing the inoculated weed species for evidence of disease; and
   (v) if disease is observed, recovering the microorganism from the diseased weed or the stock solution.

2. A microorganism having the identifying characteristics of any of those having the following identifying and deposit numbers: MB245 (NRRL B-18855), MB246 (NRRL B-18856), MB249 (NRRL B-18857), MB250 (NRRL B-18858), MB253 (NRRL B-18859), MB260 (NRRL B-18860), MB276 (NRRL B-18861), MB281 (NRRL B-18862), MB282 (NRRL B-18863), MB289 (NRRL B-18864), MB290 (NRRL B-18866).

3. A microorganism according to claim 2, having the identifying characteristics of MB245.

4. A microorganism according to claim 2, having the identifying characteristics of MB249.

5. A microorganism according to claim 2, having the identifying characteristics of MB276.

6. A method for the control of annual bluegrass, comprising application to the annual bluegrass of a microorganism according to any of claims 2 to 4.

7. A method for the control of Digitaria species (crabgrass), comprising application to the crabgrass of a microorganism according to any of claims 2 to 5.

8. A method for the control of Bromus species, comprising application to the Bromus species of a microorganism according to any of claims 2 to 4, a microorganism having the characteristics of MB218 (NRRL B-18018) or pathovar poae.

9. A m thod for control f Poa annua var. r ptans (p rennial form of Poa annua), comprising application to the Poa annua var. reptans of a microorganism acc rding to any f claims 2 t 5.

10. A m thod of treating cultivated turf grass, comprising application to the turf grass of a microorganism according t claim 2 or claim 3.

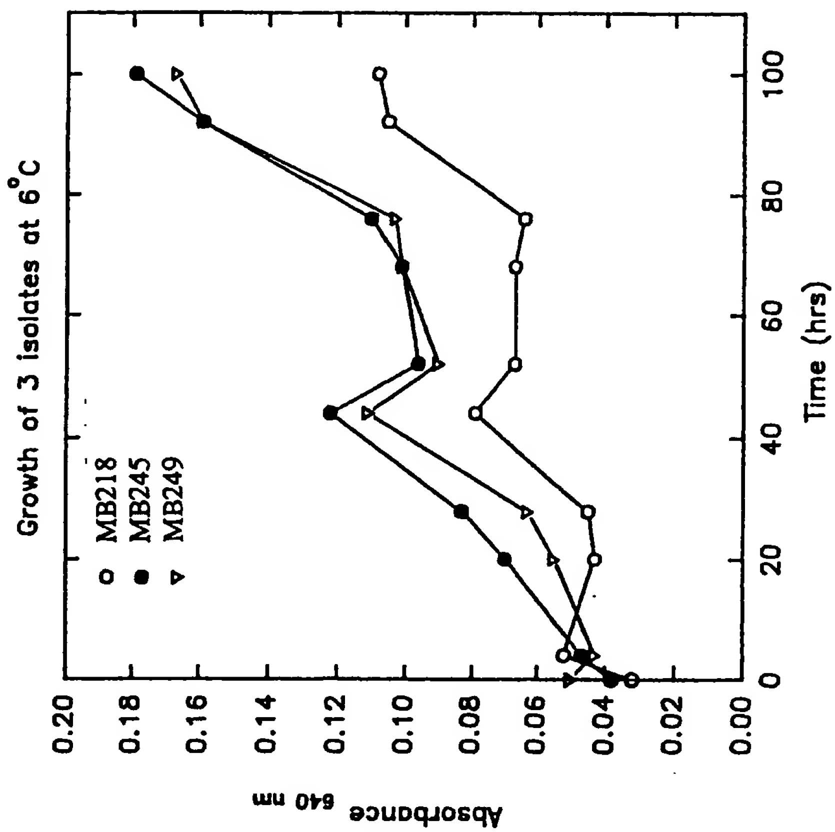
11. A m thod acc rding to any of claims 6 to 10, wh r in a plant growth regulator is also applied to th annual bluegrass, Digitaria species, Bromus species, Poa annua var. reptans, or turf grass, respectiv ly.

12. A method according t claim 11, wh rein the plant growth regulator is m fluidide.

13. A method for growing Xanthomonas in liquid culture, characterised by the growth in the culture of a microorganism according to any of claims 2 to 4..

14. A method according to claim 13, wherein the liquid culture is grown at less than or equal to ambient temperature.

FIGURE 1


Growth of 3 isolates at 6°C
MB218
MB245
MB249
Absorbance 640 nm
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
0
20
40
60
80
100
Time (hrs)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 30 7813

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	WO-A-8 801 172 (MICHIGAN STATE UNIVERSITY) * example 1 *	1-14	G01N33/569
A	EP-A-0 374 499 (MITSUI TOATSU CHEMICALS) * the whole document *	1-14	
A	PATENT ABSTRACTS OF JAPAN vol. 12, no. 172 (C-497)(3019) 21 May 1988 & JP-A-62 278 978 (DAIKIN INDUSTRIES LTD) 3 December 1987 * abstract *	1-14	
T	PHYTOPATHOLOGY vol. 81, no. 11, 1 November 1991, WASHINGTON DC USA pages 1358 - 1363 D. MING ET AL. 'Selective recovery of Xanthomas spp. from rice seeds.' * page 1358, column 1, line 3 - line 8 *	1-14	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 DECEMBER 1992	VAN BOHEMEN C.G.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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